Global Veterinaria 13 (5): 844-855, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.13.05.86223

Effect of Adding Phytase to Sheep Rations on Digestibility and Bioavailability of Phosphorus and Calcium

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Abstract: The main objective of this study was to investigate the effect of inclusion different levels of phytase enzyme in sheep rations on nutrients digestibility coefficients, nutritive value, rumen fluid parameters, nitrogen, calcium and phosphorus balances and some blood plasma parameters of mature Rahmani sheep. Ration composed of 30% Berseem hay: 70% CFM (basal diet) and the experimental groups were: basal diet with no enzyme (G_1), basal diet plus 500 IU phytase/ Kg ration (G_2) and basal diet plus 1000 IU phytase/ Kg ration (G_3). Concentrate feed mixture (CFM) composed of (46.5% yellow corn, 20% undecorticated cottonseed meal, 30% wheat bran, 1% sodium chloride, 2% limestone and 0.5% vitamins and minerals mixture). Three mature Rahmani rams were used as experimental groups using 3×3 Latin square design with three periods. The results showed that Phytase supplementation did not affect nutrient digestibility, nutritive values and ruminal total volatile fatty acids (TVFA's), ruminal ammonia nitrogen (NH_3-N), N retention for treated groups in comparison with control group (G_1). However, P and Ca balances and blood P and Ca were significantly ($P<0.05$) improved for treated groups (G_2 and G_3) compared with G_1. These results indicate that phosphorus in phytate should be considered available to adult rams. But, adding phytase increased phosphorus bioavailability and decreased fecal phosphorus excretion in those animals.

Key words: Phytase · Sheep · Phosphorus · Nutrient Digestibility

INTRODUCTION

Phosphorus is one of the major minerals needed by the body in large quantities. It is found in rocks, soils and organic materials. Approximately portion (50-75%) of total phosphorus in mature cereal grains, legumes and oilseeds is present as phytate-bound P [1]. Also known as phytic acid, it is an organic complex generally regarded as the primary storage form of phosphate in plants. Moreover, phytic acid not only reduces P availability but also other important minerals such as Ca, Mg, Zn and Fe. In addition, phytic acid complex, in a similar way, with proteins, energy and digestive enzymes including pepsin, trypsin and amylase, also making them less soluble [2-4].

Phosphorus play an important functions in the body including, cell membrane structure (Phospholipids), energy transfer, structure of nucleic acids and as an important constituent of bone, it is provided in livestock diets and therefore, it is presented in manure [5, 6].

Justification of the herd feeding programs helps to minimize environmental pollution by phosphorus excretion [7-11].

Phosphorus from phytate can be absorbed by the small intestines the phytate molecule must be hydrolyzed by the enzyme phytase. Unlike ruminants, non ruminants have a limited ability for phytase production in the digestive tract. Adeola *et al.* [2] showed that dietary supplementation with phytase in pigs resulted in linear increases in plasma Ca and P concentration. Also, phytase improved Ca and P retention.

Because ruminal microorganisms are a source of phytase for ruminants, the general assumption is that ruminants are able to utilize nearly all of the phytate P

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present in grains [1, 12, 13]. However, ruminal hydrolysis is not uniform among feedstuffs and it is affected by factors such as grain type, processing methods and ruminal outflow rates. Also, high dietary Ca reduces the effectiveness of ruminal phytase activity [14]. Moreover, the optimum pH for rumen microbial phytase was 5.5 [15]. While ruminal pH is usually between 6.0 and 7.0, which allows hydrolysis of phytate to occur but not at an optimum rate. Similarly, small amount of phytate P can escape the rumen and not be available for absorption in the intestine. Thus, phytate hydrolysis is not complete within the rumen [6].

The maintenance requirements of phosphorus in sheep is 36 mg/kg of live body weight, this requirements increases in some form of production such as growth, reproduction and lactation [16].

Under normal situation when dietary phosphorus and calcium are sufficient to meet the animal's requirement, plasma phosphorus concentration is adequate to maintain saliva concentrations and the microbial phosphorus requirement is met.

During sever deficiency; low plasma concentration will limit phosphorus supplied by saliva. A sever phosphorus deficiency will also decrease feed intake causing saliva flow to decrease as well. This scenario will limit microbial growth, decrease digestibility and volatile fatty acid production [17].

Breves and Schröder [18] noted that P depletion also reduced cellulose digestion, microbial protein synthesis and food protein degradation in the rumen. Insufficient intake of P can also affect the metabolic activity of cells in the body, which influence the satiety center, causing a decline in appetite and retardation of growth in growing animals [19].

In an effort to recycle nutrients and limit the use of commercial fertilizers, many agricultural producers have adopted the practice of applying manure to pasture and cropland. Because livestock manure is relatively rich in P when compared to the N content of the manure, relative to plant requirements, excess P is often applied to the soil [20].

Phosphorus is one of the key polluting nutrients from animal agriculture, it is an important contributor to both water and soil pollution. As the P-rich manure is continually applied to the soil and only a limited amount is removed by crops, P accumulates in the soil [21]. Once a threshold level is reached, P moves into the groundwater or flow into the surface water [11]

So this work was carried out to study the effect of adding phytase at different levels to adult sheep rations containing high concentration of phytate on digestion coefficient, ruminal fermentation and blood constituent.

MATERIALS AND METHODS

This work was carried out at the Agricultural Experimental Station, Faculty of Agriculture, Cairo University, Giza, Egypt. While, laboratory analyses were carried out at the Laboratories of Animal Production Department, National Research Centre (NRC), Dokki, Giza, Egypt.

Animals and Diets: Three mature Rahmani rams with average live body weight of 53 Kg were randomly assigned to treatments in a 3×3 Latin square design with three dietary treatments and three periods. Treatment periods lasted for 19 days; the first 14 d as an adaptation period followed by 5 d as a collection period. At the end of each period the rams were switched to a different treatment diet, therefore each ram had received all the three treatment diets. Concentrate feed mixture (CFM) composed of 46.5% yellow corn, 20% undecorticated cottonseed meal, 30% wheat bran, 1% sodium chloride, 2% limestone and 0.5% vitamins and minerals mixture.

The experimental groups were assigned as basal diet with no enzyme (G_1), basal diet plus 500 IU phytase per kilogram of diet (G_2) and basal diet plus 1000 IU phytase per kilogram of diet (G_3). The phytase enzyme was mixed with the CFM.

Management: The metabolism trials lasted for 57 days (three periods, 19 days each) in which rams were kept in separate shaded pens for the first 11 days of the adaptation period (14 d). Rams were then moved individually to the metabolism crates (which were designed to separate the feces and the urine) for the rest of the adaptation period and for 5 days of the collection period. Concentrate: Roughage ratio was 70:30 and diets were formulated to meet the ram's nutrient requirements according to the NRC [16]. Rations were offered in two portions, CFM at 8.00 am while BH was offered at 12.00 pm. Water was offered twice daily. Feed intake, water consumption, fecal and urine outputs were quantitatively collected from each animal once a day during the collection period recorded and saved for later analyses.

Table 1: Composition of the experimental groups and Chemical analyses of concentrate feed mixture (CFM), Berseem hay (BH) and the calculated composition of the basal diet (BD).

1- Experimental groups		
G_1	G_2	G_3
70% CFM + 30% B H	basal diet	basal diet
(Basal diet)	+ 500 IU Phytase	+ 1000 IU Phytase

2- Chemical analyses of concentrate feed mixture (CFM), Berseem hay (BH) and the calculated composition of the basal diet (BD)

Item (%)	CFM	BH	BD *
Dry matter (DM)	90.72	93.90	91.67
Chemical composition on DM basis			
Organic matter (OM)	89.37	88.06	88.98
Crude protein (CP)	14.15	11.80	13.45
Ether extract (EE)	3.34	2.47	3.08
Crude fiber (CF)	10.11	34.38	17.39
Nitrogen free extract (NFE)	61.77	39.41	55.06
Ash	10.63	11.94	11.02
Calcium (Ca)	0.84	0.98	0.88
Phosphorous (P)	0.50	0.28	0.43

* BD: 70% concentrate feed mixture + 30% Berseem hay.

Nitrogen, minerals (Ca & P) balances and water metabolism were determined as the difference between intake and excretion.

Composition of the experimental group diets and Chemical analyses of CFM, Berseem hay (BH) and the calculated composition of the basal diet are shown in Table (1).

Sample Collection: feces and urine samples were collected during digestibility trials. Rumen fluid samples were taken at the end of the collection period by stomach tube. Samples were collected before morning feeding, 2, 4, 6 and 8 hrs post feeding. Ruminal pH, ammonia nitrogen (NH3-N) and total volatile fatty acids (TVFA's) concentrations were determined. Ruminal pH was immediately measured using ORION RESEARCH digital pH meter, model (201). While, samples of rumen liquor were kept frozen at -20 °C until later analyses of TVFA's and NH3-N concentrations.

Blood samples were taken in 10 ml heparinized test tubes and centrifuged at 4000 r. p. m for 10 minutes then blood plasma was separated and stored frozen at -20°C for eventual analyses.

Analytical Procedures: Chemical analysis of dry matter (DM), organic matter (OM), ash, crude protein (CP), crude fiber (CF) and ether extract (EE) were determined in feeds and feces according to the Official Method of Analysis AOAC [22]. Nitrogen free extract (NFE) was calculated by the difference using the following equation: NFE = 100 – [Moisture + CP + CF + Ash + EE %].

Feed and feces samples were prepared for P and Ca determination by using the method of wet and dry digestion based on AOAC [22]. Phosphorus was determined calorimetrically using commercial kits (Quimica Clinica Aplicada S.A. Spain), according to the method of Goodwin [23]. While Ca was determined calorimetrically according to the method of Jansen and Helbing [24] using commercial kits (Quimica Clinica Aplicada S.A. Spain).

Total volatile fatty acids (TVFA's) concentrations of rumen fluid samples were determined by steam distillation according to Warner [25]. Ruminal ammonia-nitrogen (NH_3-N) concentrations were determined using kjeldahle distillation method AOAC [22].

Plasma total protein was determined calorimetrically by using commercial kits according to the method of Armstrong and Carr [26]. Albumin was determined calorimetrically by using commercial kits according to the method of Doumas *et al.* [27]. The globulin values were obtained by subtracting albumin values from total protein values. Albumin/globulin ratio (A/G) was obtained by dividing albumin value on its corresponding globulin value. Urea-nitrogen was determined calorimetrically by using commercial kits according to the method of Fawcett and Scott [28]. Glucose was determined calorimetrically by using commercial kits according to the method of Trinder [29]. Total lipids were determined calorimetrically by using commercial kits according to the method of Zollner and Kirsch [30]; (All commercial kits were purchased from Biodiagnostic. Blood plasma P was determined calorimetrically by using commercial kits (Quimica Clinica Aplicada S.A. Spain), according to the method of Goodwin [23]. Calcium was determined calorimetrically according to the method of Jansen and Helbing [24] using commercial kits (Quimica Clinica Aplicada S.A. Spain). Plasma Aspartate-Aminotransferase (AST) and Alanine-Aminotransferase (ALT) were determined calorimetrically by using commercial kits purchased from Biodiagnostic, according to the method of Reitman and Frankel [31].

Statistical Analyses: Data obtained from this study were statistically analyzed by SPSS [32]. Repeated measures ANOVA procedure was used to analyze the data of the effect of phytase supplementation with different levels and sampling time on blood and rumen parameters according to the following model:

$$Y_{ijk} = \mu + Z_i + T_j + ZT_{ij} + e_{ijk}$$

Where: Y_{ijk} = any value from the overall population; μ = the overall mean; Z_i = effect of the i^{th} phytase enzyme level; T_j = effect of the j^{th} sampling time; ZT_{ij} = effect of the interaction between the i^{th} phytase level and the j^{th} sampling time and e_{ijk}= the random error associated with the k^{th} individual receiving the i^{th} phytase level at the j^{th} sampling time.

On the other hand, Latin square design was used analyzes the data of nutrients digestibility using the general linear model (GLM) procedure according to the following model:

$$Y_{ij(k)} = \mu + T_k + R_i + C_j + e_{ij(k)}$$

Where: $Y_{ij(k)}$ = any value from the overall population; μ = the overall mean; T_k = the effect of the k^{th} treatment; R_i = the effect of the i^{th} block; C_j = the effect of the j^{th} Colum and $e_{ij(k)}$ = the experimental error.

Significant differences among treatment means were separated by Duncan's multiple range test [33] with a 5% level of probability.

RESULTS AND DISCUSSION

Chemical Composition: Chemical composition (On DM basis) of concentrate feed mixture (CFM), Berseem hay (BH) and calculated chemical composition of basal diet (BD) are shown in Table 1. Results showed that CFM contained higher level of phosphorus and lower content of calcium in comparison with BH. Calculated composition of the basal diet contained about 13.5% CP, 17.4% CF, 11% ash and 0.43% P.

Nutrient Digestibility and Nutritive Values: The effect of adding phytase enzyme to the ration of adult ruminants on nutrients digestibility and nutritive values of diets differed only in phytase level are shown in Table 2.

The digestibility of dry matter, organic matter, crude protein, ether extract, crude fiber and nitrogen free extract and the nutritive value expressed as total digestible nutrient (TDN) and digestible crude protein (DCP) were not significantly improved (P>0.05) for groups fed diets supplemented with phytase (G_2 and G_3) compared to G_1 (Table 2). However, increasing phytase level from 500 IU (G_2) to 1000 IU (G_3) improved the digestibility values but the differences were not significant.

The clarification of the mechanisms by which feed enzymes increase the digestion and the utilization of feedstuffs in ruminant rations is complicated by three main factors: 1) feeds are structurally very complex containing a variety of polysaccharides, proteins, lipids, lignin and phenolic acids often in intimate association; 2) the enzyme products are mixtures of enzymes containing many different activities each of which differs in their optimal conditions and specificities and 3) ruminal fluid is by nature an extremely complex microbial ecosystem containing hundreds of microbial species and their secreted enzymes [34, 35].

Table 2: Nutrient digestion coefficients and nutritive values of the experimental groups.

	Experimental groups			
Item	G_1	G_2	G_3	SEM
Nutrient digestion coefficients %				
DM	71.72	71.97	72.95	0.46
OM	74.36	74.64	75.62	1.23
CP	70.86	70.59	71.17	1.26
CF	60.91	61.53	61.99	1.19
EE	81.09	82.68	83.87	3.93
NFE	78.89	79.17	80.90	1.57
Nutritive values %				
TDN	69.18	69.52	70.70	1.25
DCP	9.53	9.50	9.57	0.17

G_1: 30% berseem hay + 70% CFM (basal diet) without enzyme.
G_2: basal diet + 500 IU phytase/ kg ration.
G3: basal diet + 1000 IU phytase/ kg ration.
SEM: standard error of the means.

The effect of P intake on digestibility was studied by Field *et al.* [36], who reported a decrease of DM digestibility of diets low in P. A review article by Durand and Komisarczuk [37] summarized digestibility results from various studies in which low-P diets were fed to ruminants. These studies indicated that, when determined on a mixed population *in vitro*, the rumen ecosystem appears to be phosphorus dependent for the degradation of cell wall, thus they suggested lower cell wall digestibility for low-P diets.

Shanklin [38] reported that digestibility increased in lambs fed phytic acid diets supplemented with phytase indicating that there was an increase in ruminal fluid P for these lambs which may have enhanced ruminal microorganism activity.

Nitrogen, Phosphorus, Calcium and Water Balances:

- Nitrogen Balance: Data of nitrogen balance are shown in Table 3. Nitrogen intake, excretion and retention were not significantly affected by phytase supplementation. These results are in agreement with those obtained by Guyton [39] who showed that supplementation with phytic acid had no effect on N intake, fecal N, apparent N digestibility; urinary N,

Table 3: Nitrogen, phosphorus, calcium and water balances of the experimental groups.

Item	Experimental groups			SEM
	G_1	G_2	G_3	
1- Nitrogen intake, excretion and retention (g/head/day) of the experimental groups.				
Nitrogen intake (NI)	15.68	15.68	15.87	0.34
Fecal nitrogen (FN)	4.50	4.58	4.58	0.29
Digested nitrogen (DN)	11.18	11.10	11.29	0.26
Urinary nitrogen excretion	8.59	8.69	8.62	0.52
Total nitrogen excretion	13.09	13.27	13.20	0.77
Nitrogen retention (NR)	2.59	2.41	2.67	0.23
NR % of NI	16.52	15.37	16.82	2.28
NR % of DN	23.17	21.71	23.65	2.96
2- Phosphorus intake, excretion and balance (g/ head/ day) of the experimental groups				
Phosphorus intake	3.16	3.16	3.20	0.14
Fecal excreted phosphorus	2.40[a]	1.91[b]	1.76[b]	0.16
Absorbed phosphorus	0.76[b]	1.25[a]	1.44[a]	0.17
Urinary excreted phosphorus	0.14[b]	0.27[a]	0.29[a]	0.04
Total excreted phosphorus	2.54[a]	2.18[b]	2.05[b]	0.09
Phosphorus balance	0.62[b]	0.98[a]	1.15[a]	0.10
3- Calcium intake, excretion and balance (g/ head/ day) of the experimental groups				
Calcium intake	6.43	6.43	6.51	0.28
Fecal calcium	1.29[a]	0.85[b]	0.83[b]	0.08
Absorbed calcium	5.14[b]	5.58[a]	5.68[a]	0.22
Urinary calcium excretion	0.11[b]	0.22[a]	0.24[a]	0.01
Total calcium excretion	1.40[a]	1.07[b]	1.07[b]	0.08
Calcium balance	5.03	5.36	5.44	0.21
4- *Water balance* by the experimental groups				
Drinking water, ml	5338	5257	5273	63.47
Feeds water, ml	64	67	65	2.17
Total water intake, ml/h/d	5402	5324	5338	65.56
Urinary loss, ml	2733	2903	3129	195.1
Fecal water, ml	189	164	184	25.56
Total water loss, ml/h/d	2922	3067	3313	207.6
Insensible loss, ml	2480	2257	2025	174.0

a and b Means in the same row between groups with different super scripts differ significantly (P<0.05).

SEM: standard error of means.

G_1: 30% berseem hay + 70% CFM (basal diet) without enzyme.

G_2: basal diet + 500 IU phytase/ kg ration.

G_3: basal diet + 1000 IU phytase/ kg ration.

milk N, total N excretion and N balance. On the other hand, other researchers found an effect of phytase supplementation on N balance. Shanklin [38] noted that the addition of phytase to the phytic acid diets of lambs resulted in higher N absorption compared to lambs fed the cottonseed meal supplemented with phytase. Also the same author suggested that this effect may have been due to the increase in N intake for lambs receiving the phytic acid diets supplemented with phytase. Moreover, Knowlton *et al.* [10] reported a decrease in fecal N excretion and total N excretion in cows fed diets supplemented with wheat bran, an organic P source, compared with mono-and di-calcium phosphate, an inorganic P source. This decreased N excretion was likely due to the decreased DMI in cows fed wheat bran compared with those fed mineral sources of phosphorus.

- Phosphorus Balance: Data of phosphorus balance for lambs in different experimental groups is presented in Table 3. Phytase supplementation decreased significantly (P<0.05) fecal P and total P excretion, while it improved significantly (P<0.05) the absorbed P, urinary P excretion and the P balance. Increasing phytase level from 500 IU (G_2) to 1000 IU (G_3) did not affect significantly fecal, urinary and total P excretion, absorbed P and P balance as shown in Table (3). The current results are in agreement with the findings of Satter *et al*. [6], Shanklin [38], Bravo *et al.* [40], Dilip [41], Knowlton *et al.* [42] and Knowlton *et al.* [43].

Shanklin [38] showed a possible improvement in the utilization of organic P with supplemental phytase in lambs ration. Similarly, Dilip [41] observed that the addition of exogenous phytase (427 IU/ kg total mixed ration on DM basis) significantly increased (P<0.05) total P digestibility and decreased (P<0.05) total excretion of fecal P in lactating cows fed total mixed ration containing either corn or barley. While the present study indicates a significant (P<0.05) improvement in total P absorption, P balance and a reduction in P excretion due to phytase supplementation, other researchers reported that ruminants are able to utilize nearly all of the phytate P present in grains [13].

Several factors affect the rate at which enzymatic reactions proceed: temperature, pH, enzyme concentration, substrate concentration and the presence of any inhibitors or activators [44]. In ruminants, Phytase is produced by the rumen ecosystem [15] as soon as the young ruminants start to ingest solid feed [45]. This phytase has been shown to mediate efficient use of dietary phytate P for diets containing 30% [12] and 62.5% [1] of meal concentrates. However, phytate P appears to be inefficiently absorbed by sheep when supplied in excess [46] and phytate P digestibility appears highly variable.

An explanation for this reduction in phytate P degradation with high concentrate diets would be a possible saturation of phytase activity caused by an excess phytate Pas has been shown in reports of

Satter *et al*. [6]. This might cause some phytate P to escape from the rumen before degradation and not be available for absorption in the intestine. Thus, added dietary phytase in the current study could improve the digestibility of phytate P by enhancing ruminal hydrolysis of phytate.

Another possible explanation for the enhanced hydrolysis of phytate is that the action of phytase might have occurred in the abomasum. The phytase might have quickly reached the abomasum, where the pH conditions would probably have been more favorable for phytase activity. Such action could have released phytate P that had not been released in the rumen [40].

Moreover, the concentration of Ca in the diet can affect P absorption [47] and phytate hydrolysis [48]. Although Clark *et al.* [12] observed higher apparent digestibility of P when the dietary Ca was 0.9% than 0.6%, others have found a reduction in P absorption with higher concentrations of dietary Ca [14]. Also, Barth and Hansard [49] reported that phytate P utilization was 100% when the Ca: P ratio was 2: 1, but fell to 67% when Ca: P ratio increased to 8:1. In the current study, Ca concentration was 0.88% of the diet (DM basis) and the Ca: P ratio in the diet was 2: 1.

In addition, phytase efficiency depends on the amplitude of the pH drop [50] and on the duration of low pH [51]. Both of these effects may have contributed to the difference in the effect of phytase supplementation, in the current study, between G_2 and G_3. The rumen pH measured at 2 or 4 hours post feeding corresponded roughly to the maximal efficiency of the phytase whereas the high pH in G_1 might have reduced phytase efficiency. Also, the persistence of low pH was longer in G_2 than in G_3 which extended the period of maximum efficiency of the enzyme (Table 4).

Calcium Balance: Calcium intake, excretion and balance data are shown in Table 3. Phytase supplementation decreased significantly (P<0.05) fecal Ca and total Ca excretion, while it increased significantly (P<0.05) Ca absorption and urinary Ca excretion. However, no significant changes in Ca intake or balance were observed among the experimental groups. It is apparent that increasing phytase level from 500 IU (G_2) to 1000 IU (G_3) had no significant effect (P>0.05) on absorbed Ca, fecal, urinary or total Ca excretion. These results are similar to the findings of Shanklin [38], who noticed that lambs receiving inorganic P supplementation had higher (P<0.05) urinary Ca excretion than the lambs receiving organic P supplementation. Also, the same author found that there was a phytase effect on Ca absorption when expressed in g/d, which agreed with the results of the current study.

Table 4: Rumen fluid parameters and main effects of sampling time on ruminal fluid parameters of the experimental groups.

		Experimental groups			
Item	Sampling time, hrs	G_1	G_2	G_3	SEM
pH	0	6.43	6.44	6.27	
	2	6.34	5.94	6.07	
	4	5.90	5.80	5.91	
	6	6.12	6.05	6.08	
	8	6.30	6.22	6.19	
	Overall mean	6.22	6.09	6.10	0.06
TVFA's	0	8.15	8.35	8.17	
(meq/100ml)	2	9.35	9.70	9.60	
	4	10.23	10.27	10.19	
	6	9.43	10.16	10.07	
	8	9.25	9.35	9.50	
	Overall mean	9.28	9.57	9.51	0.21
NH_3-N	0	26.50	27.00	27.50	
(mg/100ml)	2	28.33	29.00	29.17	
	4	27.93	28.17	28.50	
	6	27.00	27.50	27.67	
	8	26.75	27.15	27.60	
	Overall mean	27.30	27.76	28.09	0.68

2. Main effects of sampling time on ruminal fluid parameters of the experimental groups.

	Sampling time (hrs)					
Item	0	2	4	6	8	SEM
pH value	6.38^a	6.12^c	5.87^d	6.08^c	6.24^b	0.03
TVFA's (meq/ 100ml)	8.22^d	9.55^b	10.2^a	9.89^{ab}	9.37^c	0.15
NH_3-N (mg/ 100ml)	27.0^c	28.8^a	28.2^b	27.4^c	27.2^c	0.43

a, b, c and d Means in the same row between sampling times having different super scripts differ significantly (P<0.05).

SEM: standard error of means.

G_1: 30% Berseem hay + 70% CFM (basal diet) without enzyme.

G_2: basal diet + 500 IU phytase/ kg ration.

G_3: basal diet + 1000 IU phytase/ kg ration.

Furthermore, Challa and Braithwaite [52] found that a low P diet resulted in an increase in the fecal excretion of Ca and a decrease in Ca absorption in growing calves. In their study, Ca intake was constant with three different levels of dietary P. Also, the same authors theorized that the mechanism of this response was that as P intake declined; there was a decrease in serum P that led to less P retention by the bones. Since P was not being retained by the bones, the requirement for Ca by the skeletal tissue decreased, which led to a decrease in the absorption and retention of Ca. However, the current results are in contrast with a previous study of Dilip [41], who reported that the addition of exogenous phytase had no significant effect on fecal Ca in lactating cows fed total mixed ration containing either corn or barley.

Water Balance: There was no significant difference (P>0.05) in water metabolism between the experimental groups (Table 3).

Rumen Fluid Parameters: Data in Tables 4 represents the rumen parameters and the main effects of time on those ruminal parameters for the experimental groups. Rumen parameters are important indicators of rumen environment, microbial activity and subsequently rumen metabolism. The most indicative parameters determined in this study were rumen pH, TVFA's and ammonia nitrogen.

pH Value: Ruminal pH is one of the most critical factors affecting the fermentation and influences its functions. It varies in a regular manner depending on the nature of the diet and on the time it is measured after feeding [53].

Phytase supplementation did not significantly affect ruminal pH as shown in Table 4. However, significantly (P<0.05) the highest ruminal pH value was recorded before feeding (0 time) by rams in all groups (Table 4). It decreased significantly (P<0.05) over time until it reached its lowest level at 4 hrs post feeding (Table 4). Ruminal pH at 6 and 8 hrs post feeding, increased gradually and significantly (P<0.05) among all groups compared to the lowest, 4 hrs post feeding level.

Tripathi *et al.* [54] reported a decline in ruminal fluid pH when animals were fed a high concentrate or grain diets. Decreased rumination and salivation is normally associated with high concentrate diets [55] and the consequent lowering of the buffering capacity, coupled with rapid microbial degradation of soluble carbohydrates, results in a decline of ruminal pH.

Tripathi *et al.* [56] noticed that higher concentrate intake and low roughage intake synchronized better nutrient availability for optimum rumen fermentation and microbial growth, which in turn improved intake and nutrient digestibility.

Total Volatile Fatty Acids (TVFA's) Concentration: Factors which have been reported to affect ruminal fatty acid concentration include: p1) proportion of roughage to concentrate; 2) pelleting; 3) particle size; 4) heat treatment; 5) various oils; 6) protein level; 7) environment and 8) frequency of feeding and mineral adequacy of the diet [57]. Moreover, dry matter digestibility, rate of absorption, rumen pH, transportation of the digesta from the rumen to the other parts of the digestive tract and the microbial population in the rumen and their activities also were reported to affect TVFA's concentration in the rumen [58]. No significant effect was observed in TVFA's among all groups due to phytase supplementation (Table 4). However, as expected, data obtained indicated that TVFA's concentration followed an opposite trend of rumen pH values. The lowest total volatile fatty acids concentration levels were observed before feeding (Table 4). Gradually these value increased significantly (P<0.05) over time until it reached its highest levels at 4 hrs post feeding (Table 4).

The pattern of TVFA's levels reflects the pattern of fermentation activity in the rumen [59]. In this context similar finding were reported by Baraghit *et al*. [60]. They indicated that the progress of increasing ruminal TVFA's concentrations were paralleled with the reduction in ruminal pH. The rumen microbial production of energy is related to the fermentable materials degraded to VFA's by the rumen microorganisms during digestion [61]. Also, Fadel *et al.* [62] reported that the increasing of ruminal TVFA's concentration is an indicator for better utilization of dietary carbohydrate.

Ammonia-Nitrogen Concentration (NH_3-N): Data presented in Table 4 shows that rumen NH_3-N concentration was not significantly affected by phytase supplementation. However, rumen NH_3-N concentration, as illustrated in Table 4 indicates that the highest NH_3-N concentration was obtained at 2 hrs post feeding. Then, it decreased significantly (P<0.05) at 6 hrs post feeding among rams in all groups. The peak of ruminal NH_3-N, that was reached 2 hrs after feeding, may be due to de-amination of amino acids in the rumen as suggested by Chandra *et al*. [63]. On the other hand, Hungate [64] reported that, the main part of CP in the diet was degraded to NH_3-N in the rumen by the micro organisms and it depends to large extent on the physical and chemical nature of each protein.

Furthermore, the level of NH_3-N and TVFA's as the end products of fermentation and breakdown of dietary protein, have been used as parameters of ruminal activity by Abou-Akkada and Osman [53]. Moreover, Owen and Zinn [65] reported that declining rumen pH was associated with slow ammonia absorption. Whereas, Siddone *et al.* [66] reported that small changes in rumen pH can have marked influence on ammonia absorption from the rumen through its effect on unionized ammonia concentration.

Concentration of Inorganic Phosphorus and Calcium in Blood Plasma: Data presented in Table 5 shows the effect of phytase supplementation on plasma P_i and Ca concentration of the experimental groups.

Table 5: Plasma inorganic phosphorus and calcium concentration and Main effects of sampling time on plasma phosphorus and calcium concentration of the experimental groups.

1. Plasma inorganic phosphorus and calcium concentration of the experimental groups

Item	Sampling time, hrs	Experimental groups G_1	G_2	G_3	SEM
Phosphorus (mg/ dl)	0	4.70	5.31	5.68	
	2	5.24	6.21	6.75	
	4	4.95	5.75	6.41	
	6	4.78	5.50	5.94	
	8	4.74	5.36	5.79	
	Overall mean	4.88^b	5.63^a	6.11^a	0.27
Calcium (mg/ dl)	0	8.11	8.77	8.90	
	2	8.70	10.1	10.6	
	4	8.47	9.62	10.1	
	6	8.25	9.09	9.16	
	8	8.18	8.85	8.92	
	Overall mean	8.34^b	9.29^a	9.54^a	0.09

2. Main effects of sampling time on plasma phosphorus and calcium concentration of the experimental groups.

Item	Sampling time (hrs) 0	2	4	6	8	SEM
Phosphorus (mg/ dl)	5.23^b	6.07^a	5.70^{ab}	5.41^{ab}	5.30^{ab}	0.21
Calcium (mg/ dl)	8.59^d	9.80^a	9.39^b	8.83^c	8.65^d	0.06

a,b,c and d Means in the same row between sampling times having different super scripts differ significantly. (P<0.05)

SEM: standard error of means.

G_1: 30% berseem hay + 70% CFM (Basal diet) without enzyme.

G_2: basal diet + 500 IU phytase/ kg ration.

G_3: basal diet + 1000 IU phytase/ kg ration.

Plasma Phosphorus (P_i): Phytase supplementation increased significantly (P<0.05) plasma P_i compared to the control group (G_1). The highest P_i level was observed for G_3 (1000 IU phytase) as shown in Table (5). Plasma P_i was significantly (P<0.05) the highest 2 hrs post feeding in all groups. It decreased over time; however, the difference was not significant (Table 5). It has been reported that serum P below 4 mg/ dl indicates dietary deficiency [16]; therefore the P_i values in plasma reported for the current study are consistent with the accepted normal range of 4.0-8.0 mg/ dl [67]. Similar finding were reported by Shanklin [38], who reported that supplemented diets with organic P increased serum P. The same author stated that the range of serum P_i concentrations for lambs were from 5.12 to 8.42 mg/dl. Furthermore, Dilip [41] reported that the addition of exogenous phytase to the diet increased (P<0.05) serum P_i in cows fed diets containing barely and corn. Because blood P_i is affected by dietary P intake [68], the addition of phytase to the diets apparently increased the amount of absorbable P in the small intestine of the lambs. This could be the explanation for the increased values of plasma P_i in the current study.

Table 6: Plasma metabolites recorded for the experimental groups.

Item	Sampling time, (hrs)	Experimental groups G_1	G_2	G_3	SEM
Total protein (g/ dl)	0	5.04	5.31	5.41	
	4	5.58	5.62	5.89	
	Overall mean	5.31	5.47	5.65	0.30
Albumin (g/ dl)	0	3.43	3.47	3.55	
	4	3.45	3.51	3.59	
	Overall mean	3.44	3.49	3.57	0.06
Globulin (g/ dl)	0	1.61	1.85	1.86	
	4	2.13	2.10	2.30	
	Overall mean	1.87	1.98	2.08	0.29
A/ G ratio	0	2.21	2.08	2.01	
	4	1.75	1.72	1.59	
	Overall mean	1.98	1.90	1.80	0.27
Urea (mg/ dl)	0	45.7	46.1	46.8	
	4	46.0	46.8	47.0	
	Overall mean	45.9	46.5	46.9	1.31
Glucose (mg/ dl)	0	69.7	70.3	71.7	
	4	70.9	73.2	73.1	
	Overall mean	70.3	71.8	72.4	2.18
Total lipids (mg/ dl)	0	449	457	478	
	4	502	499	506	
	Overall mean	476	478	492	16.2
ALT (units/ ml)	0	15.0	14.3	15.5	
	4	15.5	14.8	15.8	
	Overall mean	15.3	14.6	15.7	0.48
AST (units/ ml)	0	22.5	21.5	22.0	
	4	23.5	22.7	24.3	
	Overall mean	23.0	22.1	23.2	0.76

G_1: 30% Berseem hay + 70% CFM (basal diet) without enzyme.

G_2: basal diet + 500 IU phytase/ kg ration.

G_3: basal diet + 1000 IU phytase/ kg ration.

SEM: standard error of the means.

Plasma Calcium: Phytase supplementation significantly (P<0.05) increased plasma Ca compared to the control group (G_1) (Table 5). Increasing phytase level from 500 IU (G_2) to 1000 IU (G_3) did not significantly (P<0.05) affect plasma Ca (Table 6). The highest (P<0.05) Plasma Ca levels were observed at 2 hrs post feeding in all groups. It decreased significantly (P<0.05) over time. The lowest (P<0.05) levels were obtained before feeding (zero time) and for 8 hrs post feeding (Table 5).

The plasma Ca levels reported in the current study are consistent with the accepted normal range of 8.0-12.0 mg/dl [38]. These results are in agreement with the finding of Field *et al.* [36]; they reported that serum Ca levels were greater than 8 mg/ dl for lambs receiving adequate dietary Ca. Similarly, Shanklin [38] reported that serum Ca was higher (P<0.04) for lambs fed phytic acid compared to lambs fed cottonseed meal diets. However, the current results are in contrast to the finding of Dilip [41] who

reported that the concentration of Ca in cows serum were not affected either by the exogenous phytase or the grain source.

Blood Plasma Parameters: Data presented in Table 6 shows the effect of phytase supplementation on blood plasma metabolites for the experimental groups. Blood metabolites (Total protein, albumin, globulin, A/G ratio, urea, glucose, total lipids, ALT and AST) were not significantly affected by phytase supplementation among all groups. These results are similar to the result reported by Shanklin [38], who noticed that phytase supplementation had no effect on the blood urea nitrogen of lambs fed either cottonseed meal or phytic acid diets.

CONCLUSION

From the results obtained from this study it could be concluded that phytase supplementation did not improve nutrient digestion coefficients or nutritive value. However, adding phytase increased phosphorus bioavailability and decreased fecal phosphorus excretion therefore decreased the environmental pollution with P from farm animals.

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